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Conventional and Molecular Identification of Culturable Airborne Bacteria

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Abstract

Bacteria isolated from the environment during the present study were representative of normal microflora of the skin, respiratory and urinary tracts; it also includes some soil and water-borne pathogenic and nonpathogenic genera. Six samples from different locations were studied for bacterial investigation. Among 14 isolates obtained, 13 were Gram positive, and the rest one was Gram negative. Out of 13 Gram positive isolates, 12 were round-shaped non spore forming and were identified as *Planococcus citreus, Stomatococcus mucilaginosus*, *Kocuria kristinae, Micrococcus agilis* (2), *Kytococcus sedentarius* (2), *Micrococcus luteus*, *Micrococcus lylae* and *M. roseus*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and rod-shaped non spore forming identified as *Renibacterium salmoninarum.* The Gram-negative bacteria was identified as *Pseudomonas aeruginosa.* Other than provisional identification, two isolates (JG 40 and SG 49) were further confirmed through molecular characterization on the basis of 16Sr RNA gene sequence analysis as *Staphylococcus aureus* and *Pseudomonas aeruginosa* repectively. Spearman's correlation showed that air temperature and wind speed negatively correlated with the bacterial abundance. It is clear that none of the samples containing airborne pathogens collected was safe for human health due to presence of potentially pathogenic microorganisms. Many were human pathogenic as well as food poisoning micro-organisms.

Introduction

Humans are intimately related to the air. Microorganisms disperse widely in the aerosphere (Griffin 2007, Burrows et al. 2009a) with thousands to millions of cells per cubic meter of air (Lighthart 2000) and consist of a wide range of taxa in urban environments (Brodie et al. 2007, Bowers et al. 2011a). Most of the airborne bacteria

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originate from natural sources such as soils, lakes, oceans, animals and men (Proctor 1935, Zobell 1946). Bacteria enter the near-surface atmosphere by continuous aerosolization from various surfaces such as those from plants, animals (including humans), soil and water (Burrows et al. 2009b, Harrison 2004). These bacterial communities have been associated with various processes, being causative agents of human health (Douwes et al. 2003) and even influencing atmospheric conditions (Christner et al. 2008, Bowers et al. 2009). The study of these bio-aerosols, has been identified as a high priority and immediacy issue concerning not only public health but also climate, environment, ecology, epidemiology and environmental engineering (Peccia et al. 2008).

The culturable atmospheric bacteria diversity is generally dominated by Gram positive bacteria, particularly the phyla Firmicutes (e.g. *Bacillus* and *Staphylococcus* spp.) and Actinobacteria (e.g. *Micrococcus* spp.) (Després et al. 2012, Gandolf et al. 2013, Lighthart 2000).Some studies showed higher concentrations of cocci due to lower susceptibility of organisms to environmental stress due to the presence of pigments and higher peptidoglycan in their cell wall preventing them from drying and heat stress (Raymond et al. 2001).

Outbreaks of the foot and mouth disease and Q fever in England have been linked to the aerosolization of pathogens originating from diseased livestock (Donaldson and Alexandersen 2002, Smith et al. 1993, Hawker et al. 1998). For instance, outdoor airborne bacteria and fungi can cause several types of respiratory illnesses or conditions such as asthma, bronchitis, pneumonia, chronic obstructive pulmonary disease COPD, seasonal allergies, and others (Yassin and Almouqatea 2010, Bowers et al. 2011b). This is also illustrated by a recent report of the World Health Organization (WHO 2018), globally, 4.2 million deaths are attributable to ambient air pollution (AAP) in 2016. About 91% of these deaths occur in low- and middle-income (LMI) countries.

The relationship between aerial microbial community composition and meteorology is still not well characterized (Gunthe et al. 2016). Even bacteria within the same structural classification (e.g. Gram negative) may vary in how they respond to temperature and relative humidity (Tang 2009).

The present study was aimed at investigating the abundance, diversity and meteorological effects on airborne bacteria of a coastal urban area of Dhaka city near Buriganga River.

Materials and Methods

In all six air samplings were conducted during November, 2017 to May, 2018 from rooftop of Zoology Department of Jagannath University (S1), Jagannath University gate (S2), Bahadur Shah Park (S4), Sadarghat Gate-10 (S4), Ahsan Manzil (S5) and Mitford Ghat (S6). Sterile plates with different media, estio HTC-1 Digital Hygrometer Thermometer, LX-1330 Digital Illuminance meter, camera, watch, markers, field

notebook, and stand were taken to the sampling sites. For all samplings, data from 3 meteorological parameters (air temperature, relative humidity and wind speed) were measured.

Four ~~Five~~ types of media (each deployed in duplicate during each exposure) were used. Airborne bacteria were collected by using settling plate technique (Brown 1953). Nutrient agar (NA) medium was used for the enumeration and isolation of aerobic heterotrophic bacteria, while Baird parker agar (Difco), Mannitol salt agar (Diagnostic Pasteur), Cetrimide agar (Difco) were used for the determination and isolation of pathogenic bacteria present in air samples.

All the culture plates were incubated at 37°C in dark for 48 hrs. Bacterial colony counting was made with the help of a digital colony counter (DC-8 OSK 100086, Kayagaki, Japan). Discrete bacterial colonies were isolated immediately after counting. In Baird Parker Agar medium, colonies appeared black and shiny, with a fine white rim, surrounded by a clear zone which was expected to be *Staphylococcus aureus* a human pathogenic bacterium. In Mannitol salt agar non-mannitol fermenters such as *S. epidermidis,* show pinkish colonies with no yellow color change in the medium. In Cetrimide agar medium, green colonies were considered *Pseudomonas aeruginosa,* a human pathogenic bacterium. The selected colonies were marked and given a unique identifier and studied for various characteristics *viz.* color, form, elevation, margin surface, optical characters etc. (Eklund and Lankford 1967, Bryan 1950). . During this investigation a total of 75 isolates were primarily selected. Finally, 15 isolates were chosen randomly and purified for further essential study for identification.

Following Bergey's Manual (Sneath et al. 1986) the physiological and biochemical tests of the isolated bacteria were carried out. Considering the physiological and biochemical characteristics of the bacterial isolates, provisional identification was made with the help of laboratory manuals (Sneathet al. 1986, Krieg and Holt 1984).

Two conventionally identified isolates were subjected to molecular identification on the basis of 16S rDNA sequence analysis for further confirmation. The following primer pairs were used - 5'-16S rRNA: CCAGACTCCTACGGGAGGCAGC, 3'-16S rRNA: CTTG-TGCGGGCCCCCGTCAATTC for the partial amplification of 16S rRNA gene. The supernatant of heat lysed cell suspension was used as the source of template DNA during PCR amplification of the 16S rRNA gene. The PCR reaction was performed following an initial denaturation at 95°C for 5 min, denaturation at 94°C for 1 min, annealing at 55°C for 30 sec, extension at 72°C for 1 min, and final extension was at 72°C for 10 min. After completion of the cycling program, the reactions were held at 4°C. The amplified products were separated electrophoretically on 1% agarose gel. DNA bands were observed on UV-transilluminator and photographed by a gel documentation system (Microdoc DI-HD, MUV21-254/365, Cleaver Scientific). The sequence generated from automated sequencing of PCR products was analyzed through NCBI-BLAST database (http://blast. ncbi.nlm.nih.gov/) and rRNA BLAST (http://bioinformatics.

psb.ugent.be/cgi-bin/rRNA/ blastform.cgi) programs to find out possible similar organism through alignment of homologous sequences.

To find out the relationship between environmental factors and airborne bacteria, non-parametric test Spearman correlation was applied. The analysis was performed using SPSS 22.0 (IBM, USA).

Result and Discussion

Some biochemical characteristics and provisionally identified isolates are shown in Table 1. Considering the morphological, cultural and biochemical characters, selected isolates were provisionally identified. Out of 14 isolates, 13 were Gram positive, and one was Gram negative bacteria. Among 13 Gram positive isolates, *Micrococcus* was the dominant genus. Under the genus *Micrococcus,* the provisionally identified species were *Kocuria kristinae*, *Micrococcus agilis* (2), *Kytococcus sedentarius* (2), *Micrococcus luteus*, *Micrococcus lylae*, *Micrococcus roseus* and other five were *Planococcus citreus*, *Stomatococcus mucilaginosus*, *Renibacterium salmoninarum*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. The Gram negative isolates was identified as *Pseudomonas aeruginosa*. Besides, two isolates were subjected to molecular characterization. The average number of bacteria from two replicates exposed in different culture media for 30 min, and the meteorological parameters along with the study period were presented in Fig. 2. The air temperature ranged between 24.6 and 32°C. The relative humidity ranged from 34.5 to 83%. Bacterial abundance is shown in Table 2. The Gram positive *Micrococcus* was the dominating member (53.34%) followed by *Staphylococcus* (13.34%).

In the approximate gel size of the amplified DNA band was observed as 600 bp (Fig. 1). The amplified DNA was sequenced, and through NCBI-BLAST and rRNA BLAST analysis the isolate JG-40 was identified as *Staphylococcus cohnii* and the isolate SG-49 was identified as *Pseudomonas aeruginosa* (Table 3).

Spearman's correlation showed that air temperature and wind speed negatively correlated with the bacterial abundance of Baired Parker Agar ($r = -0.829$) and bacterial colony that formed in mannitol salt agar ($r = -0.812$), respectively. In addition, the positive correlation ($r = 0.841$) was observed between air temperature and wind speed. So, both environmental factors can influence the abundance of airborne bacteria negatively.

Previous studies have shown that temperature above 24°C appeared to universally decrease airborne bacterial survival (Tang 2009) which clearly support the result of the present study in which bacterial concentration was highest in S2 site where temperature was lowest (24.6°C) and the negative correlation of bacteria with temperature.

The relative humidity has a negative correlation with bacterial concentrations. Studies on airborne Gram negative bacteria such as *Serratiamarcescens*, *Escherichia coli*, *Salmonella pullorum*, *Salmonella derby*, *Pseudomonas aeruginosa* and *Proteus vulgaris* have

Table 1. Result of biochemical tests and provisional identification of the selected bacterial isolates.

Isolate No.	Motility	Oxidase	Catalase	Methyl red	Voges-Proskauer	Indole production	Starch hydrolysis	Citrate utilization	Gelatin liquefaction	Provisional identification
ZR-10	–	+	+	–	+	–	–	+	–	*Kocuria kristinae*
ZR-17	–	–	+	–	+	+	–	–	–	*Staphylococcus epidermidis.*
SG-31	+	+	+	–	–	+	–	+	+	*Kytococcus sedentarius*
SG-35	–	+	+	–	+	–	–	+	–	*M. lylae*
SG-20	+	+	+	–	–	–	+	–	–	*M. luteus*
SG-49	+	+	+	–	–	–	–	+	+	*Pseudomonas aeruginosa*
BP-18	+	–	+	+	+	+	+	–	+	*M. agilis.*
JG-15	–	+	+	–	–	–	–	+	–	*Kytococcus sedentarius*
JG-40	+	–	+	+	+	–	–	+	+	*Staphylococcus aureus.*
AM-11	+	–	+	–	+	–	–	–	+	*Planococcus citreus*
AM-24	–	+	+	+	–	–	–	+	+	*Stomatococcus mucilaginosus*
AM-16	–	–	+	–	+	–	+	–	+	*M. roseus*
MG-21	+	–	+	+	–	–	+	+	+	*M. agilis.*
MG-33	–	+	+	–	+	–	–	+	+	*Renibacterium salmoninarum*

Table 2. Culturable airborne bacterial abundance in the Old Dhaka city near Sadarghat area.

Name of organism	Number of occurrences	Abundance (%)
Gram positive (round shaped non-spore former)		
Micrococcus	8	57.15 ~~53.34~~
Staphylococcus	2	14.29 ~~13.34~~
Planococcus	1	7.15 ~~6.67~~
Stomatococcus	1	7.15 ~~6.67~~
Gram-positive (rod-shaped non-spore former)		
Renibacterium	1	7.15 ~~6.67~~
Gram negative		
Pseudomonas	1	7.15 ~~6.67~~

Table 3. Conventional and molecular identification of two selected isolates.

Isolate name	Conventional name	Molecular identification				
		Scientific name	Strain	Strain identity match (%)	Max. coverage score	E-value
JG-40	*Staphylococcus aureus*	*Staphylococcus cohnii*	AS21	94	994	0.0
SG-49	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	HOB1	99	1000	0.0

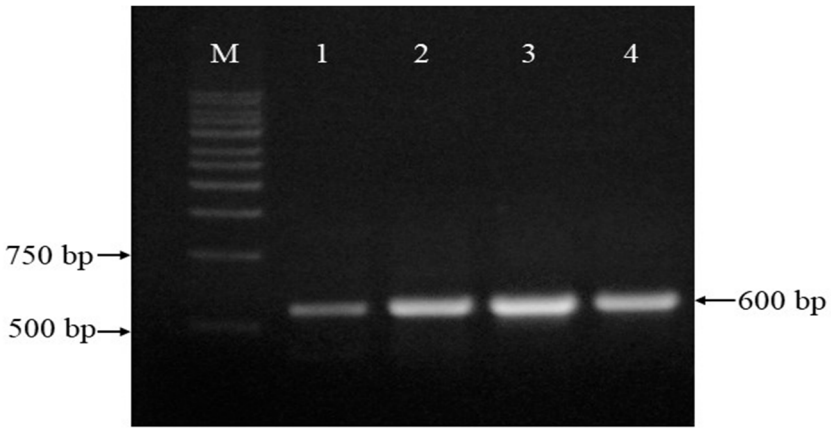

Fig. 1. PCR amplification of part of the 16S rRNA gene. Lane M = 1.0 kb ladder, lanes 1-2 represent isolate SG49 and lanes 3-4 represent isolate JG40. Approximate size of the amplified DNA band was 600 bp.

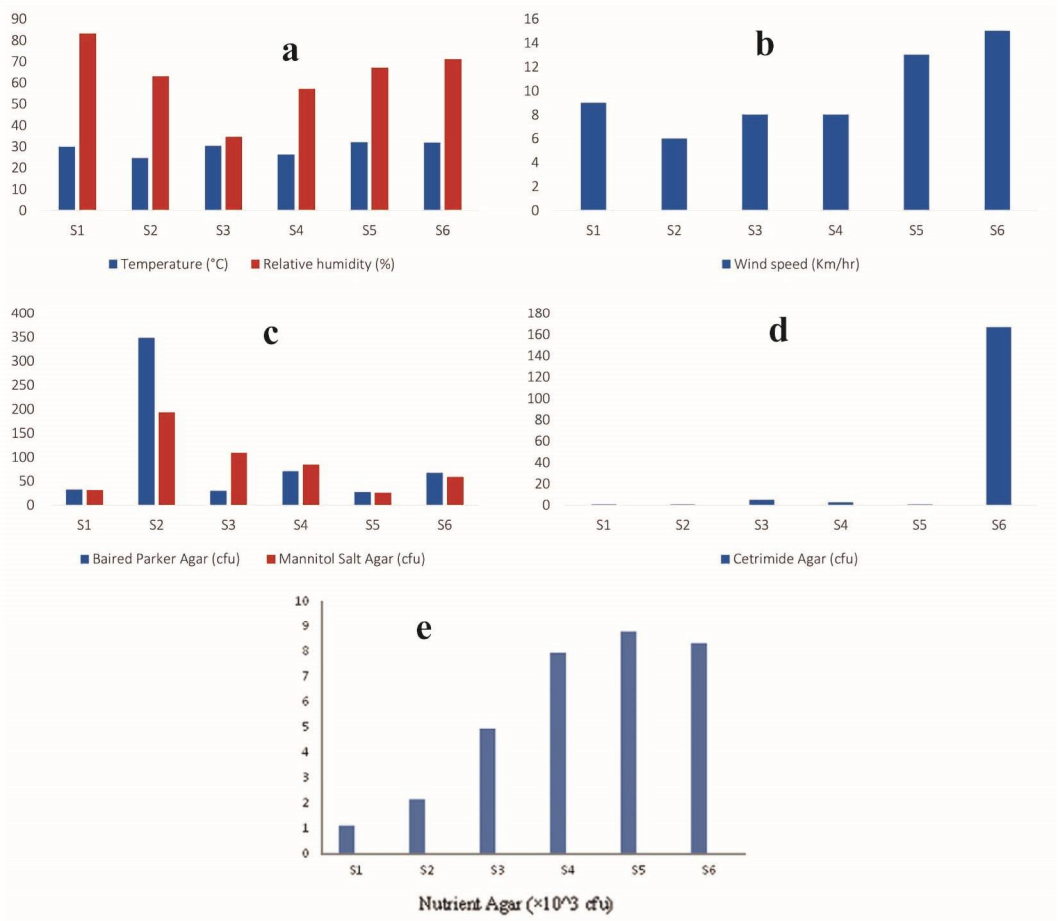

Fig. 2. Different environmental parameters (a-b) and abundance of bacteria (c, d and e) in different media after exposure of 30 min.

increased death rates at intermediate (approx. 50 - 70%) to high (approx. 70 - 90%) RH environments (Webb 1959, Won and Ross 1966). For some airborne Gram positive bacteria, *Staphylococcus albus*, *Streptococcus haemolyticus*, *Bacillus subtilis* and *Streptococcus pneumoniae* (type 1), their death rates were also highest at intermediate RH levels (Dunklin and Puck 1948, Webb 1959, Won and Ross 1966).

Pseudomonas aeruginosa is responsible for 10 - 15% of the nosocomial infections worldwide (Blanc et al. 1998). It may be a resident in the gut in healthy people. Eight per cent of healthy individuals carried *P. aeruginosa* in stool samples in a study conducted in Spain (Estepa et al. 2014).

Table 4. Spearman's correlations (Sampling number, N = 6) of environmental and biological data.

		Temp. (°C)	Relative humidity (%)	Wind speed (km/hr)	Nutrient agar (cfu)	Cetrimide agar (cfu)	Baired parker agar (cfu)	Mannitol salt agar (cfu)
Temp. (°C)	Correlation coefficient	1.000	0.257	**0.841***	–0.029	0.273	**–0.829***	–0.714
	Sig. (2-tailed)		0.623	0.036	0.957	0.600	0.042	0.111
Relative humidity	Correlation coefficient	0.257	1.00	0.638	–0.086	–0.273	–0.086	–0.657
	Sig. (2-tailed)	0.623	0	0.173	0.872	0.600	0.872	0.156
Wind speed	Correlation coefficient	**0.841***	0.638	1.00	–0.116	0.308	–0.522	**–0.812***
	Sig. (2-tailed)	0.036	0.173		0.827	0.553	0.288	0.050

*Correlation is significant at the 0.05 level (2-tailed).

This study will generate available information about the load, type and meteorological effects on of bacteria associated with the air from polluted locations. Among the bacteria, found in the present investigation *Staphylococcus aureus, Pseudomonas aeruginosa, Kocuria kristinae*, *Micrococcus luteus and M. lylae* were reported as pathogenic to human.

References

Blanc DS, Petignat C, Janin B, Bille J and **Francioli P** (1998) Frequency and molecular diversity of *Pseudomonas aeruginosa* upon admission and during hospitalization: A prospective epidemiologic study. Clin. Microbiol. Infect **4**: 242-247.

Bowers RM, Lauber CL, Wiedinmyer C, Hamady M, Hallar AG, Fall R, Knight R and Fierer N (2009) Characterization of airborne microbial communities at a high-elevation site and their potential to act as atmospheric ice nuclei. Appl. Environ. Microb. **75**(15): 5121-5130.

Bowers RM, **Sullivan AP**, **Costello EK**, **Collett JL**, **Knight R** and **Fierer N** (2011a) Sources of Bacteria in Outdoor Air across Cities in the Midwestern United States. Applied and Environmental Microbiology **77**(18): 6350-6356.

Bowers RM, McLetchie S, Knight R, Fierer N (2011b) Spatial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments. ISME J. **5**(4): 601-612.

Bryan AH (1950) Manual of methods for pure culture study of bacteria. McGraw Hill Book Co. Inc., NY. 12(1): Leaflet. I-X.

Burrows SM, Elbert W, Lawrence MG and **PoschlU** (2009a) Bacteria in the global atmosphere-Part 1: review and synthesis of literature data for different ecosystems. Atmospheric Chemistry and Physics Discussion **9**(3): 9263-9280.

Burrows SM, Butler T, Jöckel P, Tost H, Kerkweg A, Pöschl U and **Lawrence MG** (2009b) Bacteria in the global atmosphere-Part 2: Modeling of emissions and transport between different ecosystems. Atmos. Chem. Phys. **9**(23): 9281-9297.

Christner BC, Cai R, Morris CE, McCarter KS, Foreman CM, Skidmore ML, Montross SN and **Sands DC** (2008) Geographic, seasonal and precipitation chemistry influence on the abundance and activity of biological ice nucleators in rain and snow. P. Natl. Acad. Sci. U.S.A. **105**: 18854-18859.

Després VR, Huffman JA, Burrows SM, Hoose C, Safatov AS, Buryak G, Nowoisky JF, Elbert W, Andreae MO, PöschlU and **Jaenicke R** (2012) Primary biological aerosol particles in the atmosphere: A review. Tellus B. **64**: 1-58.

Donaldson AI and **Alexandersen S** (2002) Predicting the spread of foot and mouth disease by anairborne virus. Rev. Sci. Tech. **21**(3): 569-575.

Douwes J, Thorne P, Pearce N and **Heederik D** (2003) Bioaerosol health effects and exposure assessment: Progress and prospects. Ann. Occup. Hyg. **47**: 187-200.

Dunklin EW and **Puck TT** (1948) The lethal effect of relative humidity on air borne bacteria. J. Exp. Med. **87**: 87-101.

Eklund C and **CE Lankford** (1967) Laboratory Manual for General Microbiology. Prentice-Hall, Inc. Englewood Cliffs, New Jersey. pp. 52-55.

Estepa V, Rojo-Bezares B, Torres C and **Sáenz Y** (2014) Faecal carriage of *Pseudomonas aeruginosa* in healthy humans: Antimicrobial susceptibility and global genetic lineages. FEMS Microbiol. Ecol. **89**(1): 15-19.

Gandolfi I, Bertolini V, Ambrosini R, Bestetti G and **Franzetti A** (2013) Unravelling the bacterial diversity in the atmosphere. Applied microbiology and biotechnology. **97**(11): 4727-4736.

Griffin DW (2007). Atmospheric movement of microorganisms in clouds of desert dust and implications for human health. Clinical Microbiology Reviews **20**(3): 459-477.

Gunthe SS, Hoor P, Burrows SM, Kampf CJ, Lang-Yona N and **Després VR** (2016) Bioaerosols in the earth system: Climate, health, and ecosystem interactions. Atoms. Res. **182**: 346-376.

Harrison RM, Jones AM, Biggins, PDE, Pomeroy N, Cox CS, Kidd SP, Hobman JL, Brown NL, and **Beswick A** (2004) Climate factors influencing bacterial count in background air samples. Int. J. Biometeorol. **49**: 167-178.

Hawker JI, Ayres JG, Blair I, Evans MR, Smith DL, Smith EG, Burge PS, Carpenter MJ, Caul EO, Coupland B, Desselberger U, Farrell ID, Saunders PJ, Wood MJ (1998) A large outbreak of Q fever in the West Midlands: windborne spread into a metropolitan area? Commun Dis Public Health **1**(3): 180-187.

Tang JW (2009) The effect of environmental parameters on the survival of airborne infectious agents, J. R. Soc. Interface **6**: S737-S746.

Kellogg CA and **Griffin DW** (2006) Aerobiology and the global transport of desert dust. Trends Ecol. Evol. **21**: 638-644.

Krieg NR and **Holt JG** (1984) Bergey'sManual of Systematic Bacteriology. Vol. 1. Williams and Wilkins Company, Baltimore. pp. 964.

Lighthart B (2000). Mini-review of the concentration variations found in the alfresco atmospheric bacterial populations. Aerobiologia **16**: 7-16.

Peccia J, Milton DK, Reponen T and **Hill J** (2008) A role for environmental engineering and science in preventing bioaerosol-related disease. Environmental Science and Technology **42**(13): 4631-4637.

Proctor BE (1935) The microbiology of the upper air. J. Bacteriol. **30**: 363-375.

Raymond D, Pelletier SJ, Crabtree TD, Schulman AM, Pruett TL and **Sawyer RG** (2001). Am. Surg. **67**(9): 827-832.

Smith DL, Ayres JG, Blair I, Burge PS, Carpenter MJ, Caul EO, Coupland B, Desselberger U, Evans M, Farrell ID, Hawker JI, Smith EG and **Wood MJ** (1993) A large Q fever outbreak in the West Midlands: clinical aspects. Respiratory Medicine 87(7): 509-516.

Sneath PHA, Mair, Sharpe ME and **Holt JG** (1986) Bergey's Manual of Systematic Bacteriology (9th ed). Williams and Wilkins Company, Baltimore. London. Vol. 2,

Webb SJ(1959) Factors affecting the viability of air-borne bacteria. I. Bacteria aerosolized from distilled water. Can. J. Microbiol. **5**: 649-669.

WHO (2018) Burden of disease from ambient air pollution for 2016 (Vol. II). World Health Organization, Geneva. pp. 1-4.

Won WD and **Ross H** (1966). Effect of diluent and relative humidity on apparent viability of air borne *Pasteurella* pestis . Appl. Microbiol. **14**: 742-745.

Yamamoto N, Bibby K, Qian J, Hospodsky D, Rismani-Yazdi H, Nazaroff WW and **Peccia J** (2012) Particle-size distributions and seasonal diversity of allergenic and pathogenic fungi in outdoor air. ISME Journal. **6**: 1801-1811.

Yassin MF and **Almouqatea S** (2010) Assessment of airborne bacteria and fungi in an indoor and outdoor environment. Int. J. Environ. Sci. Technol. **7**(3): 535-544.

Zobell CE and **Mathews HM** (1936) A qualitative study of the bacterial flora of sea and land breezes. Proc. Natl. Acad. Sci. U.S.A. **22**: 567-572.

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